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Expression of α_2-macroglobulin receptor/low density lipoprotein receptor-related protein is increased in reactive and neoplastic glial cells

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Abstract

α_2-Macroglobulin receptor/low density lipoprotein receptor-related protein (α_2M-R/LRP) is a multi-functional cell-surface receptor that has been implicated in diverse physiologic processes In normal human brain, α_2M-R/LRP is expressed principally by pyramidal neurons with localization to cell bodies and proximal processes By contrast, α_2M-R/LRP is not present in either the cell bodies or processes of most normal macroglia (including astrocytes) In this investigation, we studied the expression of α_2M-R/LRP in the brain, following tissue injury or neoplastic transformation, by immunohistochemistry Significantly increased α_2M-R/LRP immunoreactivity was identified in reactive astrocytes, indicating that expression of this receptor is regulated in vivo in response to brain injury α_2M-R/LRP immunoreactivity was also detected in glial cell tumors, this finding is novel since malignant transformation is typically thought to turn off expression of this receptor

Key words α_2-Macroglobulin receptor, Low density lipoprotein receptor-related protein, Tumor invasion, Glioma

1. Introduction

α_2-Macroglobulin receptor/low-density lipoprotein receptor-related protein (α_2M-R/LRP) is a multi-functional plasma membrane receptor which has been implicated in cellular lipoprotein metabolism, cell-surface trafficking of plasminogen activators and their inhibitors, modulation of growth factor responses, and *Pseudomonas* exotoxin A clearance [1–6] α_2M-R/LRP is synthesized as a 600 kDa precursor protein which undergoes post-translational processing into 515-kDa and 85-kDa subunits [7,8]. The 515-kDa subunit confers ligand binding activity and is entirely extracellular The 85-kDa subunit has a transmembrane domain that anchors the large subunit to the cell surface by a non-covalent interaction [8,9] α_2M-R/LRP and its mRNA are distributed through numerous human tissues [7,10], however, the cellular distribution in human brain is selective Immunoreactivity is restricted almost exclusively to neurons, principally of the pyramidal type in the cerebral cortex, subcortical gray matter, and cerebellum [10,11] Although α_2M-R/LRP is not normally expressed by most glia, immunoreactivity is detected in a highly localized perivascular zone which corresponds to the distribution of astrocytic foot processes. Expression of α_2M-R/LRP in this region suggests a role for the receptor in the function of the blood–brain barrier

Expression of α_2M-R/LRP is regulated in vitro by cytokines and mediators of inflammation In macrophages, α_2M-R/LRP is up-regulated by colony stimulating factor-1 and down-regulated by interferon-γ and bacterial lipopolysaccharide [12,13] Based on work with fibroblasts in culture, Van Leuven et al [14] suggested that cancer cells may express greatly decreased levels of α_2M- R/LRP. More recently, we demonstrated that α_2M-R/ LRP is decreased or absent in hepatocellular carcinomas, as determined by immunohistochemistry [15]

Following brain injury or after neoplastic transformation, astrocytes undergo profound phenotypic changes, associated with increased proliferation and an augmented capacity for tissue invasion The purpose of the present investigation was to determine whether brain injury or glial neoplastic transformation is associated with altered α_2M-R/LRP expression

2. Materials and methods

2 1 *Materials*

Fifty-seven glioma specimens from archives of the University of Virginia Health Sciences Center were examined The tumors were classified according to the World Health Organization (WHO) criteria [16] Histopathological examination revealed 4 low-grade astrocytomas, 2 gemistocytic astrocytomas, 11 anaplastic astrocytomas, 13 glioblastomas multiforme, 2 gliosarcomas, 13 pilocytic astrocytomas, 1 pleomorphic xanthoastrocytoma, 1 optic glioma, 3 oligodendrogliomas, and 7 ependymomas Reactive (non-neoplastic) astrocytes were studied in 5 specimens with acute and chronic infarcts and in the tissue adjacent to tumors

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2 2 Immunohistochemistry

Monoclonal antibody 8G1 directed against the α_2M-R/LRP 515-kDa chain was kindly provided by Dr Dudley K Strickland (American Red Cross, Rockville, MD) 8G1 was incubated with formalin-fixed, deparaffinized sections for 15–18 h at 4°C The primary antibody was detected using the avidin-biotin immunoperoxidase technique [11] Normal non-immune serum was substituted for primary antibody as a negative control Sections of normal human liver were treated in parallel to provide positive controls

3. Results and discussion

In contrast with quiescent astrocytes in normal brain, activated astrocytes in the gray and white matter were consistently immunoreactive for α_2M-R/LRP The pattern of immunoreactivity varied from predominantly perinuclear, punctate accumulations, to a more diffuse cytoplasmic distribution The first pattern (Fig 1, arrowheads) was highly suggestive of an intracytoplasmic distribution while the diffuse form (Fig 1, inset) could reflect a combination of both surface and cytoplasmic localization The cytoarchitectural distribution of α_2M-R/LRP in reactive astrocytes was clearly different from the restricted pattern of immunoreactivity in perivascular astrocytic processes, reported previously [11]. Activated astrocytes were the only reactive glial cells with increased α_2M-R/LRP Myelin-forming oligodendrocytes and ependymal cells, which lined the ventricles in zones contiguous with injured brain, did not show α_2M-R/LRP immunoreactivity.

Neoplastic transformation of macroglial cells resulted in increased expression of α_2M-R/LRP antigen, irregardless of cell type (ie astrocytic, oligodendroglial or ependymal) (Table 1); however, the level and cytoarchitectural distribution of α_2M-R/LRP in the different tumors was variable (Table 2). Astrocytomas which diffusely invade surrounding brain are shown in Fig 2A–C In many of the tumor cells, α_2M-R/LRP immunoreactivity was conspicuously localized in the cytoplasm as punctate, perinuclear accumulations (panels A and C). Other tumor cells with larger amounts of cytoplasm showed more diffuse immunoreactivity (Fig 2B and 2C inset) In contrast to the invasive astrocytomas, pilocytic astrocytomas, which have limited capacity to invade, expressed α_2M-R/LRP primarily in cell processes (Fig 2D)

Table 1
α_2M-R/LRP immunohistochemistry in gliomas

Tumor	Cases (positive/total)
Astrocytoma	4/4
Gemistocytic astrocytoma	2/2
Anaplastic astrocytoma	11/11
Glioblastoma multiforme	12/13
Pleomorphic xanthoastrocytoma	1/1
Pilocytic astrocytoma	12/13
Optic glioma	1/1
Oligodendroglioma	3/3
Ependymoma	7/7

Most neoplastic oligodendroglial cells (Fig 3) and ependymal cells (not shown) expressed α_2M-R/LRP, in contrast with their non-malignant (normal and reactive) counterparts, which were immunonegative The pattern of immunoreactivity in the oligodendrogliomas varied Most cells expressed α_2M-R/LRP in a punctate, perinuclear pattern, similar to that observed with reactive and neoplastic astrocytes (panel A) Other cells demonstrated α_2M-R/LRP immunopositivity in a more diffuse pattern (panel B); however, this distribution was not common

The results reported in this investigation provide sharp

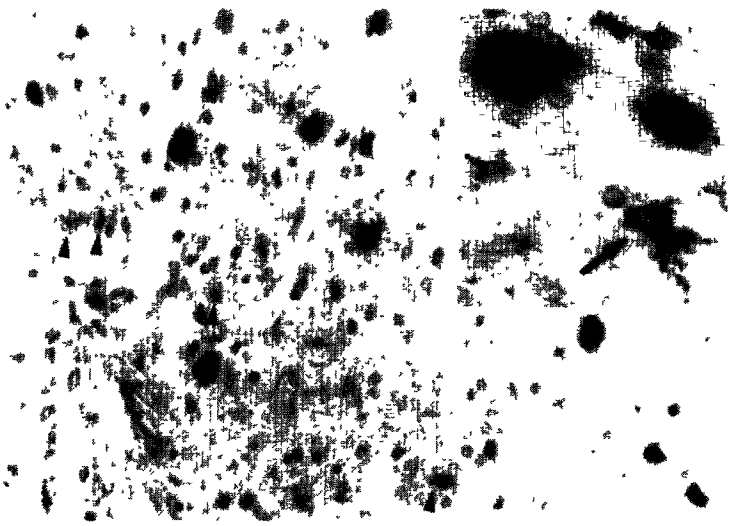

Fig 1 Localization of α_2M-R/LRP in a brain infarct Reactive astrocytes on the periphery of the lesion (left) demonstrated cytoplasmic α_2M-R/LRP immunoreactivity in both a punctate (small astrocytes, arrowheads) and diffuse (inset gemistocytic astrocytes, upper, and fibrillary astrocyte, arrow) pattern Fibrillary astrocytes with prominent staining in cellular processes were present in small numbers (inset, arrow) [Monoclonal antibody 8G1 immunoperoxidase, DAB with light hematoxylin counterstain, × 300, × 600 inset]

Table 2
Pattern of α_2M-R/LRP immunoreactivity in glial cells

Cytoplasmic distribution	
Reactive astrocytes	punctate (circumscribed) > diffuse
Neoplastic macroglia	punctate (circumscribed) > diffuse
Cellular processes	
Reactive astrocytes	rare
Infiltrating astrocytomas	rare
Circumscribed astrocytomas	common
Oligodendrogliomas	rare
Ependymomas	rare
Cell surface	
Reactive astrocytes	none
Astrocytomas	present
Oligodendrogliomas	present
Ependymomas	none

contrast with our previous study of normal human brain [11] Expression of α_2M-R/LRP in reactive and transformed astrocytes suggests that these cells synthesize α_2M-R/LRP in response to physiologic signals in vivo. The predominant intracytoplasmic localization of α_2M-R/LRP in reactive and neoplastic macroglia was surprising since α_2M-R/LRP is a plasma membrane receptor. Moestrup et al. [17] reported a similar finding in their analysis of fibroblasts and attributed the intracytoplasmic distribution of α_2M-R/LRP to intracellular vesicles in the receptor recycling pathway.

The function of α_2M-R/LRP in normal brain is still unknown, however, proteinases, proteinase inhibitors and cytokines are important effectors of glial-neuronal cell interactions. Based on its ability to bind diverse ligands (some of which are carriers of other macromole-

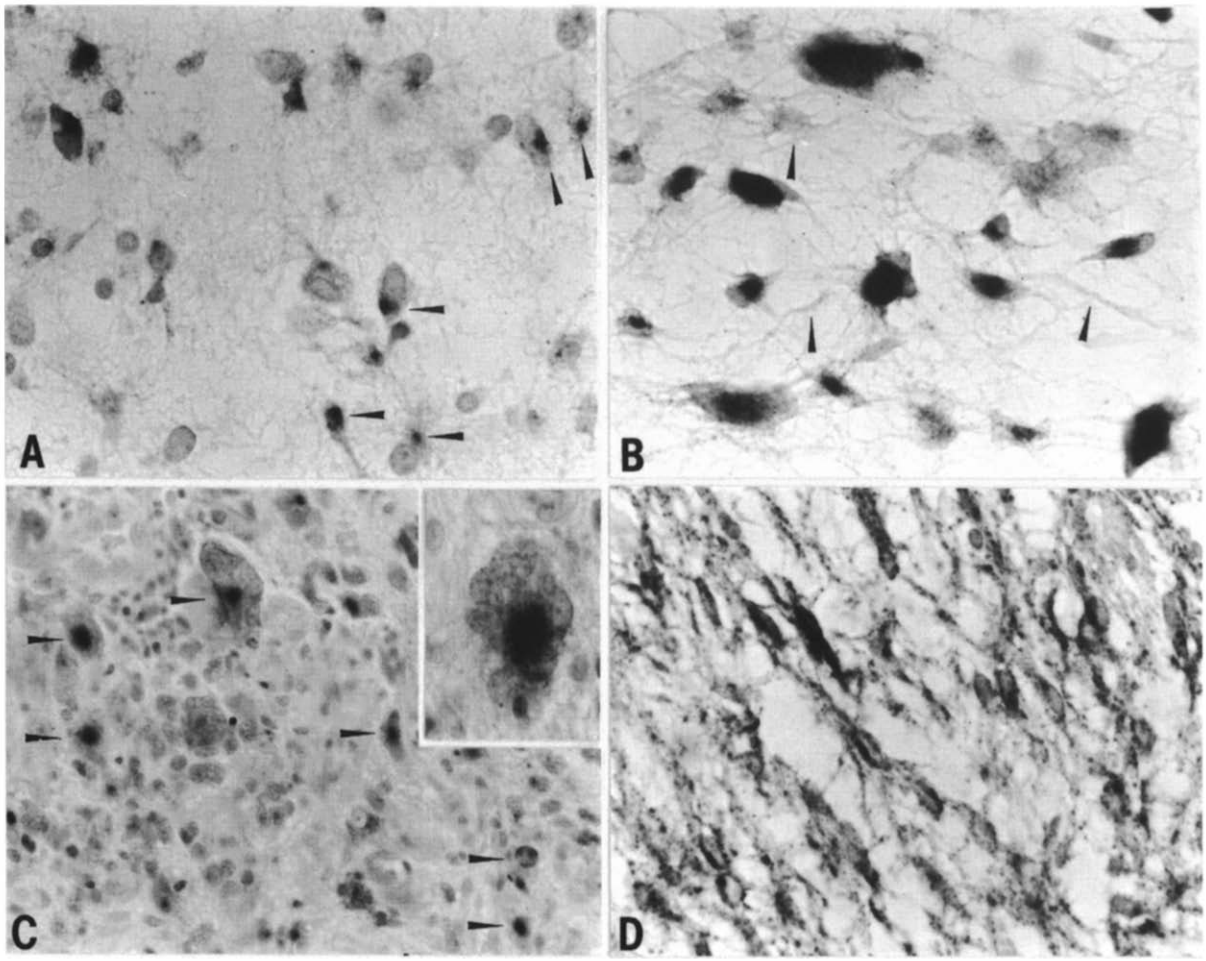

Fig 2 Localization of α_2M-R/LRP in astrocytic tumors (A–C) α_2M-R/LRP immunoreactivity was detected in all grades of diffusely infiltrating astrocytomas (A) α_2M-R/LRP immunoreactivity in low-grade astrocytomas was localized in the cytoplasm as punctate staining (arrowheads) in the perinuclear region (× 600) (B) Tumor cells with increased cytoplasmic size (gemistocytic astrocytes) usually had a more diffuse pattern of staining Note that the fine fibrillary processes (arrowheads) of the tumor cells are not labelled (× 600) (C) Cells in glioblastoma multiforme also demonstrated a punctate pattern of immunostaining for α_2M-R/LRP (arrowheads) while the increased cytoplasm of giant, multinucleated cells was more diffusely immunoreactive (× 300, × 600 inset) (D) Pilocytic astrocytomas demonstrated a striking difference of α_2M-R/LRP distribution, in contrast to the diffusely invasive astrocytic tumors, with immunoreactivity localized only to cell processes Note the fine particulate pattern of the staining in the cellular processes (× 600) (Monoclonal antibody 8G1 immunoperoxidase, DAB with hematoxylin counterstain)

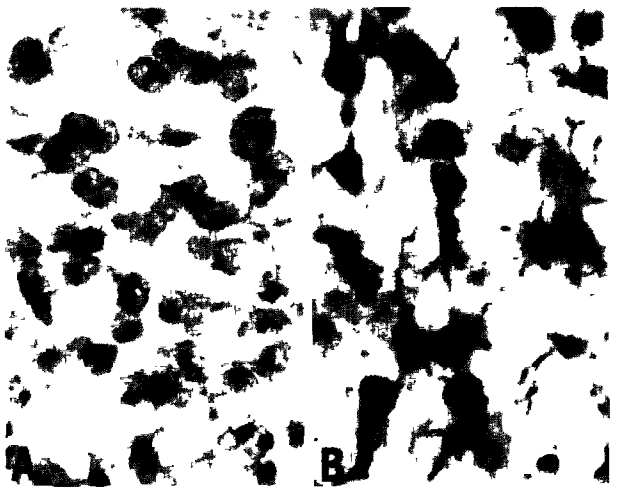

Fig 3 Localization of α_2M-R/LRP in oligodendrogliomas (A) α_2M-R/LRP immunoreactivity was conspicuously localized as punctate, perinuclear accumulations in the scant cytoplasm (B) In focal areas of the same tumor, blunt cellular processes and plasma membrane were also highlighted by the immunostaining (× 600) (8G1 immunoperoxidase)

cules), α_2M-R/LRP may function in the transfer of critical effectors between cells in the brain For example, cytokines, such as transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF), bind to activated α_2M which is a ligand for α_2M-R/LRP Since cytokine binding does not inhibit the interaction of α_2M with its receptor, these complexes can be targeted to cells which express α_2M-R/LRP [5,18] In the brain, PDGF is important in gliogenesis [19] PDGF also supports proliferation of reactive astrocytes following brain injury [20] PDGF and TGF-β influence the growth of malignant gliomas [21,22] Since α_2M is present in developing brain [23] and in adult brain during subacute responses to injury and chronic degenerative states [24,25], α_2M may influence the trafficking of TGF-β and PDGF between cells in the brain The ultimate delivery of α_2M-TGF-β or α_2M-PDGF complex to a particular brain cell depends on expression of α_2M-R/LRP Disease processes which increase α_2M-R/LRP expression in glial cells might facilitate α_2M-targeting of TGF-β and PDGF to these cells

Binding of ligands to α_2M-R/LRP results in receptor clustering in clathrin-coated pits and rapid clearance of the receptor-ligand complexes from the cell surface by endocytosis [26] The ligands are transferred to lysosomes while the α_2M-R/LRP typically recycles to the cell surface [27] Although all types of gliomas exhibited α_2M-R/LRP immunoreactivity, the more invasive astrocytomas demonstrated a predominantly cytoplasmic pattern of α_2M-R/LRP staining while the less invasive pilocytic astrocytomas expressed α_2M-R/LRP primarily in cell processes Pilocytic astrocytomas express high levels of proteinase inhibitors compared with other glial cell neoplasms [28] Whether expression of proteinase inhibitors and α_2M-R/LRP supports the non-invasive phenotype of the pilocytic astrocytoma is a topic for future investigation

In summary, the present study has demonstrated that expression of α_2M-R/LRP is regulated in glial cells in vivo Regulated expression and selective cellular localization of α_2M-R/LRP in reactive and neoplastic glia suggests a role for this receptor in glial cell function

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